SHORT COMMUNICATIONS

Porcine Epidemic Diarrhea Virus and Porcine Deltacoronavirus not Detected in Waterfowl in the North American Mississippi Migratory Flyway in 2013

Sarah W. Nelson,[1] **Michele M. Zentkovich,**[1] **Jacqueline M. Nolting,**[1] **and Andrew S. Bowman**[1,2]   [1]The Ohio State University, Department of Veterinary Preventive Medicine, 1920 Coffey Road, Columbus, Ohio, USA; [2]Corresponding author (email: bowman.214@osu.edu)

ABSTRACT: Cloacal swab samples collected from 538 migratory waterfowl along the Mississippi Migratory Bird flyway in 2013 were tested for porcine epidemic diarrhea virus and porcine deltacoronavirus. Neither virus was detected in any of the samples, indicating that waterfowl likely did not contribute to the rapid spread of these viruses within central US.

   Key words: Coronavirus, disease outbreaks, ducks, North America, swine, wild.

Porcine epidemic diarrhea virus (PEDv) has resulted in millions of swine deaths in North America since its first detection in the US during April 2013 and in Canada during January 2014 (Mole 2013; Cima 2014; Kochhar 2014). This highly contagious coronavirus causes severe diarrhea, dehydration, and vomiting in pigs of all ages, but it is notorious for high mortality of neonatal piglets. While new to North America in 2013, PEDv was already a global pathogen, and the PEDv isolates initially detected in the US closely resembled PEDv strains in China during 2011 and 2012 (Huang et al. 2013). Once in North America, the virus quickly spread during 2013 and 2014 (Chen et al. 2014), which cost the US pork industry an estimated USD$1 billion (Weng et al. 2016).

Following the introduction of PEDv, the first detection of porcine deltacoronavirus (PDCoV) in US swine occurred in February 2014, with PDCoV quickly being detected in several states (Ma et al. 2015). Similar to PEDv, PDCoV causes diarrhea, vomiting, and dehydration in swine, but is characterized as having a lower mortality rate than PEDv (Jung et al. 2016). While PDCoV was first detected in piglets in China in 2004, birds (families Anatidae, Ardeidae, Estrildidae, Muscicapidae, Passeridae, Pycnonotidae, Rallidae, Turdidae, Zosteropidae) serve as hosts for a number of highly similar deltacoronaviruses (Woo et al. 2009, 2012; Jung et al. 2016).

While feedbags and other fomites were indicated to be the primary route of PEDv and PDCoV introduction into the US (Scott 2016), one hypothesis for the rapid spread of these viruses across the country was that wild birds were facilitating transmission between farms. This could have occurred by birds serving as mechanical vectors and carrying virus with them, or birds could have acted as biologic vectors by becoming infected with the virus and shedding it in feces (Beam et al. 2015). Birds could easily contaminate feed, farm environments, or equipment, which would allow infection of pigs in confined swine production facilities. This hypothesis is supported by the ability of European Starlings (*Sturnus vulgaris*) to shed transmissible gastroenteritis virus (TGEv), another enteric swine coronavirus, for up to 32 h post exposure (Pilchard 1965). Additionally, birds carrying TGEv have caused outbreaks in swine facilities (Cooper 2000). Therefore, we tested historic samples collected for influenza A virus surveillance in waterfowl for PEDv and PDCoV.

Because the majority of swine production occurs within the Mississippi River watershed, we focused on waterfowl moving along the Mississippi Migratory Bird flyway, the most-utilized migratory bird flyway in the US (Bellrose and Kortright 1976). We selected 538 cloacal swabs collected from 12 January 2013 to 30 December 2013 from states and providences with documented swine enteric coronavirus diseases (Table 1).

An animal use protocol was not needed to collect samples from hunter-harvested bird

TABLE 1. Geographic and temporal distribution of 538 cloacal swabs collected from wild waterfowl in the Mississippi Migratory Bird Flyway during 2013 that were tested for porcine epidemic diarrhea virus and porcine deltacoronavirus.

	January	February	March	April	May	June	July	August	September	October	November	December	Totals
Arkansas	–[a]	–	–	–	–	–	–	–	–	–	–	15	15
Illinois	3	–	–	–	–	–	–	–	10	60	34	9	116
Indiana	–	–	–	–	–	–	–	–	–	60	–	–	60
Iowa	–	–	–	–	–	–	–	–	6	–	–	–	6
Maryland	–	–	–	–	–	3	5	12	4	–	–	4	28
Mississippi	12	–	–	–	–	–	–	–	–	–	–	57	69
Missouri	–	–	–	–	–	–	–	–	8	–	29	29	66
Ohio	–	23	1	–	–	–	7	17	14	21	24	9	116
Ontario	–	–	–	–	–	–	–	–	–	10	12	–	22
Wisconsin	–	–	–	–	–	–	–	–	25	7	8	–	40
Totals	15	23	1	0	0	3	12	29	67	158	107	123	538

[a] (–) = Not sampled.

carcasses, and scientific collection permits were obtained from local or state authorities where required for the original avian influenza surveillance effort. Samples were held on dry ice during transport to the lab and stored at −80 C until tested. Samples were thawed and inoculated into embryonating chicken eggs in an attempt to isolate IAV. Samples were refrozen and stored at −80 C until RNA could be extracted. The samples were tested for PEDv and PDCoV using real-time reverse transcriptase PCR (RRT-PCR). The RNA was purified using a commercial kit (Omega Bio-tek, Norcross, Georgia, USA) according to protocol (Spackman 2008). Following extraction, the RNA was subjected to RRT-PCR targeting the PEDv nucleoprotein gene (Jung et al. 2014). Using the same RNA, on the same day samples were also tested for PDCoV using RRT-PCR targeting the membrane gene segment (Wang et al. 2014). Cut points for both assays were calculated by setting the threshold at 5% of the respective positive control (RNA extracted from confirmed PEDv and PDCoV isolates) at cycle 40; samples with a cycle threshold value of ≤40 were considered positive.

Samples from birds in Ohio and Illinois accounted for the largest share of the samples in this study (Table 1). Cloacal swabs were tested from 21 avian species: Mallard (*Anas platyrhynchos*; n=141), American Green-winged Teal (*Anas crecca carolinensis*; n=69), Wood Duck (*Aix sponsa*; n=60), Blue-winged Teal (*Anas discors*; n=58), Northern Shoveler (*Anas clypeata* ; n=56), Gadwall (*Anas strepera*; n=40), Ring-necked Duck (*Aythya collaris*; n=18), Lesser Scaup (*Aythya affinis*; n=17), Redhead (*Aythya americana*; n=17), American Wigeon (*Anas americana*; n=11), Northern Pintail (*Anas acuta*; n=10), Bufflehead (*Bucephala albeola*; n=6), Canada Goose (*Branta canadensis*; n=6), American Black Duck (*Anas rubripes*; n=5), Common Goldeneye (*Bucephala clangula*; n=5), Greater Scaup (*Aythya marila*; n=5), Ruddy Duck (*Oxyura jamaicensis*; n=4), Hooded Merganser (*Lophodytes cucullatus*; n=3), Mallard × American Black Duck hybrid (*Anas rubripes* × *Anas platyrhynchos* hybrid;

n=2), Canvasback (*Aythya valisineria*; *n*=2), Snow Goose (*Chen caerulescens*; *n*=1), and the environment (*n*=2). Of the 529 ducks sampled, 452 (85%) were dabbling ducks with the remaining 77 (15%) being diving ducks. While IAV was recovered from 43 of the samples in this subset, all 538 were negative for PEDv and PDCoV.

Wild waterfowl could have visited farms with infected swine or eaten contaminated feed, but our data do not support the hypothesis that waterfowl contributed to the rapid spread of PEDv within the US. Only 38 (7%) of our samples were collected prior to the first PEDv detection in the US, with the rest collected after 30 April 2013. The PEDv was actively spreading in pigs during the study period; the US Department of Agriculture (USDA) reported 66 PEDv positive accessions from Illinois, 57 from Ohio, 17 from Missouri, five from Indiana, three from Wisconsin, one from Maryland, and 59 from Iowa during the period when avian samples were also collected from each respective state (USDA 2014).

There was no detection of PDCoV in US pigs until February 2014, after our samples were collected (Ma et al. 2015). The data presented here do not support the hypothesis that waterfowl contributed to the spread of PDCoV either. We acknowledge the relatively small sample size of our study; however, because PEDv was being concurrently detected in pigs in the same states where these wild migratory waterfowl were sampled, we would have expected to find some evidence of PEDv in wild birds if they were involved in the viral transmission (Mole 2013; Stevenson et al. 2013; USDA 2014). Nonwaterfowl species, especially peridomestic species that were not assessed in this study, could potentially play a role in viral transmission between swine production facilities.

The risk of PEDv and PDCoV exposure and transmission likely differs among North American duck species due to differences in their preferred habitat and feeding habits (e.g., dabbling ducks versus diving ducks). Although wild waterfowl typically utilize wetland and marsh habitats, native habitat destruction and abundant food sources on agricultural farms have resulted in an increased observation of waterfowl on farms (Fox et al. 2017). Stopover sites are crucial for migratory birds to rest and refuel during their journey, and they spend approximately 90% of the migration time at successive stopover locations resting and foraging along their route (Hedenstrom and Alerstam 1998). Stopover time can vary from a few hours to days depending on the species and availability of food and shelter at a location. Because grain used for swine feed is commonly transported on inland waterways such as the Mississippi River and its subsidiaries, there is ample opportunity for contamination of feedstuffs by wild birds during transportation (Fuller and Yu 2004). Additionally, open, uncovered storage of grain can create opportunities for birds and other wildlife to consume and contaminate future swine feed inputs. While PEDv and PDCoV were not detected in the birds included in this study, active bird and wildlife deterrents in open grain storage settings may be warranted, not only for preservation of product but also for disease prevention.

LITERATURE CITED

Beam A, Goede D, Fox A, McCool MJ, Wall G, Haley C, Morrison R. 2015. A porcine epidemic diarrhea virus outbreak in one geographic region of the United States: Descriptive epidemiology and investigation of the possibility of airborne virus spread. *PLOS ONE* 10:e0144818.

Bellrose FC, Kortright FH. 1976. *Ducks, geese & swans of North America*: A completely new and expanded version of the classic work by FH Kortright. 2ndEd.Stackpole Books, Harrisburg, Pennsylvania, 543 pp.

Chen Q, Li G, Stasko J, Thomas JT, Stensland WR, Pillatzki AE, Gauger PC, Schwartz KJ, Madson D, Yoon KJ, et al. 2014. Isolation and characterization of porcine epidemic diarrhea viruses associated with the 2013 disease outbreak among swine in the United States. *J Clin Microbiol* 52:234–243.

Cima G. 2014. PED virus reinfecting US herds. Virus estimated to have killed 7 million-plus pigs. *J Am Vet Med Assoc* 245:166–167.

Cooper VL. 2000. Diagnosis of neonatal pig diarrhea. *Vet Clin North Am Food Anim Pract* 16:117–133.

Fox AD, Elmberg J, Tombre IM, Hessel R. 2017. Agriculture and herbivorous waterfowl: A review of the scientific basis for improved management. *Biol Rev Camb Philos Soc* 92:854–877.

Fuller S, Yu T. 2004. Briefing paper on the upper Mississippi and Illinois Rivers transportation corridors: Grain transportation rates and associated market area. *FAPRI-UMC Briefing Paper #05-04*, Food and Agricultural Policy Research Institute, University of Missouri Columbia, Columbia, Missouri, 22 pp.

Hedenström A, Alerstam T. 1998. How fast can birds migrate? *J Avian Biol* 29:424–432.

Huang YW, Dickerman AW, Piñneyro P, Li L, Fang L, Kiehne R, Opriessnig T, Meng XJ. 2013. Origin, evolution, and genotyping of emergent porcine epidemic diarrhea virus strains in the United States. *MBio* 4:e00737–13.

Jung K, Hu H, Saif LJ. 2016. Porcine deltacoronavirus infection: Etiology, cell culture for virus isolation and propagation, molecular epidemiology and pathogenesis. *Virus Res* 226:50–59.

Jung K, Wang Q, Scheuer KA, Lu Z, Zhang Y, Saif LJ. 2014. Pathology of US porcine epidemic diarrhea virus strain PC21A in gnotobiotic pigs. *Emerg Infect Dis* 20:662–665.

Kochhar HS. 2014. Canada: Porcine epidemic diarrhea in Canada: An emerging disease case study. *Can Vet J* 55:1048–1049.

Ma Y, Zhang Y, Liang X, Lou F, Oglesbee M, Krakowka S, Li J. 2015. Origin, evolution, and virulence of porcine deltacoronaviruses in the United States. *MBio* 6:e00064–15.

Mole B. 2013. Deadly pig virus slips through US borders. *Nature* 499:388.

Pilchard EI. 1965. Experimental transmission of transmissible gastroenteritis virus by starlings. *Am J Vet Res* 26:1177–1179.

Scott A, McCluskey B, Brown-Reid M, Grear D, Pitcher P, Ramos G, Spencer D, Singrey A. 2016. Porcine epidemic diarrhea virus introduction into the United States: Root cause investigation. *Prev Vet Med*. 123:192–201.

Spackman E. 2008. A brief introduction to the avian influenza virus. *Methods Mol Biol* 436:1–6.

Stevenson GW, Hoang H, Schwartz KJ, Burrough ER, Sun D, Madson D, Cooper VL, Pillatzki A, Gauger P, Schmitt BJ, et al. 2013. Emergence of porcine epidemic diarrhea virus in the United States: Clinical signs, lesions, and viral genomic sequences. *J Vet Diagn Invest* 25:649–654.

USDA (United States Department of Agriculture). 2014. *Swine enteric coronavirus disease testing summary report*. https://www.aphis.usda.gov/animal_health/animal_dis_spec/swine/downloads/SECD_pre-fed_order_nahln_test_sum_rpt.pdf. 52 pp. Accessed Month XXXX.

Wang L, Byrum B, Zhang Y. 2014. Detection and genetic characterization of deltacoronavirus in pigs, Ohio, USA, 2014. *Emerg Infect Dis* 20:1227–1230.

Weng L, Weersink A, Poljak Z, De Lange K, Von Massow M. 2016. An economic evaluation of intervention strategies for porcine epidemic diarrhea (PED). *Prev Vet Med* 134:58–68.

Woo PC, Lau SK, Lam CS, Lai KK, Huang Y, Lee P, Luk GS, Dyrting KC, Chan KH, Yuen KY. 2009. Comparative analysis of complete genome sequences of three avian coronaviruses reveals a novel group 3c coronavirus. *J Virol* 83:908–917.

Woo PC, Lau SK, Lam CS, Lau CC, Tsang AK, Lau JH, Bai R, Teng JL, Tsang CC, Wang M, et al. 2012. Discovery of seven novel mammalian and avian coronaviruses in the genus *Deltacoronavirus* supports bat coronaviruses as the gene source of *Alphacoronavirus* and *Betacoronavirus* and avian coronaviruses as the gene source of *Gammacoronavirus* and *Deltacoronavirus*. *J Virol* 86:3995–4008.